HYBRIDIZATION OF GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE*

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1. Introduction

Hybridization studies with native glyceraldehyde-3-P dehydrogenase [1, 2] have resulted primarily in the association of dimeric subunit pairs into an R_2Y_2 intermediate R_2Y_2 = a symmetrical hybrid tetramer between the rabbit muscle ($R_4 \rightleftharpoons 2\ R_2$) and yeast ($Y_4 \rightleftharpoons 2\ Y_2$) enzymes. This work, combined with others [3–5] suggests that, functionally, the enzyme is a 'dimer of dimers'. Most of the mammalian muscle glyceraldehyde-3-P dehydrogenases studied thus far have essentially identical electrophoretic migration patterns and this precludes studies on hybrid formation. Meighen and Schachman [6] were able to chemically modify subunits of glyceraldehyde-3-P dehydrogenase, altering the electrophoretic mobility, and enabling hybridization studies to be carried out between modified and native subunits. This study is the only case in which a complete hybrid set of 5 isoenzymes has been obtained in vitro with mammalian glyceraldehyde-3-P dehydrogenase. However, Lebherz et al. [7] recently demonstrated that complete hybridization between rabbit and salmon glyceraldehyde-3-P dehydrogenases can be obtained in the presence of adenine nucleotides.

We have shown that glyceraldehyde-3-P dehydrogenase from the muscle of the parasitic roundworm, *Ascaris suum*, is electrophoretically distinct from the enzyme of mammalian sources in that it exhibits an anodic migration at pH 8.6 [8]. We have purified this enzyme from the muscle tissue of *Ascaris* (Kochman et al., unpublished results) and have demonstrated that it is very similar in structure and composition to the rabbit muscle enzyme [9]. In the present study, the purified ascarid glyceraldehyde-3-P dehydrogenase was hybridized under conditions of low protein concentration and low ionic strength with the enzyme from human, pig and rabbit muscles. In each case a complete 5 membered hybrid set of isoenzymes was formed via a symmetrical As_2X_2 intermediate.

2. Experimental procedure

2.1. *Enzymes*

Human muscle glyceraldehyde-3-P dehydrogenase was purified using the procedure of Baranowski and Wolny [10], pig muscle glyceraldehyde-3-P dehydrogenase was purified by the method of Elödi and Szöreney [11], and rabbit muscle glyceraldehyde-3-P dehydrogenase was obtained according to Kochman

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and Rutter [12]. The ascarid enzyme was isolated by a modification of Kochman and Rutter's method [12].

2.2. *Electrophoresis*

Zone electrophoresis on cellulose polyacetate strips (Gelman Instrument Co.) was performed in 0.05 M barbital (pH 8.6) at 280 V for 90 min at 2–4°C. The point of application of the enzyme was equidistant from the electrodes. Glyceraldehyde-3-P dehydrogenase activity on the strips was revealed by the staining procedure described by Lebherz and Rutter [13]. Protein was stained with 1% Amido Black 10 B in 7% acetic acid.

2.3. *Hybridization*

Prior to use, glyceraldehyde-3-P dehydrogenase crystals were dissolved and dialyzed in the following buffer: 10 mM Tris, pH 8.7; 5 mM sodium tetraborate; 0.2 mM EDTA, and 10 mM 2-mercaptoethanol. The protein samples were then diluted with this buffer to 1.0 mg protein per ml. Equimolar amounts of the diluted solutions of two parental glyceraldehyde-3-P dehydrogenases (ascarid + rabbit, ascarid + pig, or ascarid + human) were mixed and again dialyzed against the same amount of buffer with continuous stirring. Aliquots of 4 μl of the mixture were taken for electrophoretic analysis. In control experiments, diluted samples of glyceraldehyde-3-P dehydrogenase were dialyzed separately against the above buffer. All the hybridization experiments were performed at 4–6°C.

3. Results and discussion

As shown in fig. 1, in all cases after a prolonged incubation (24 hr) of the mixture of the two types of glyceraldehyde-3-P dehydrogenases, the five-membered interspecies hybrid set was formed: As_4, As_3X, As_2X_2,

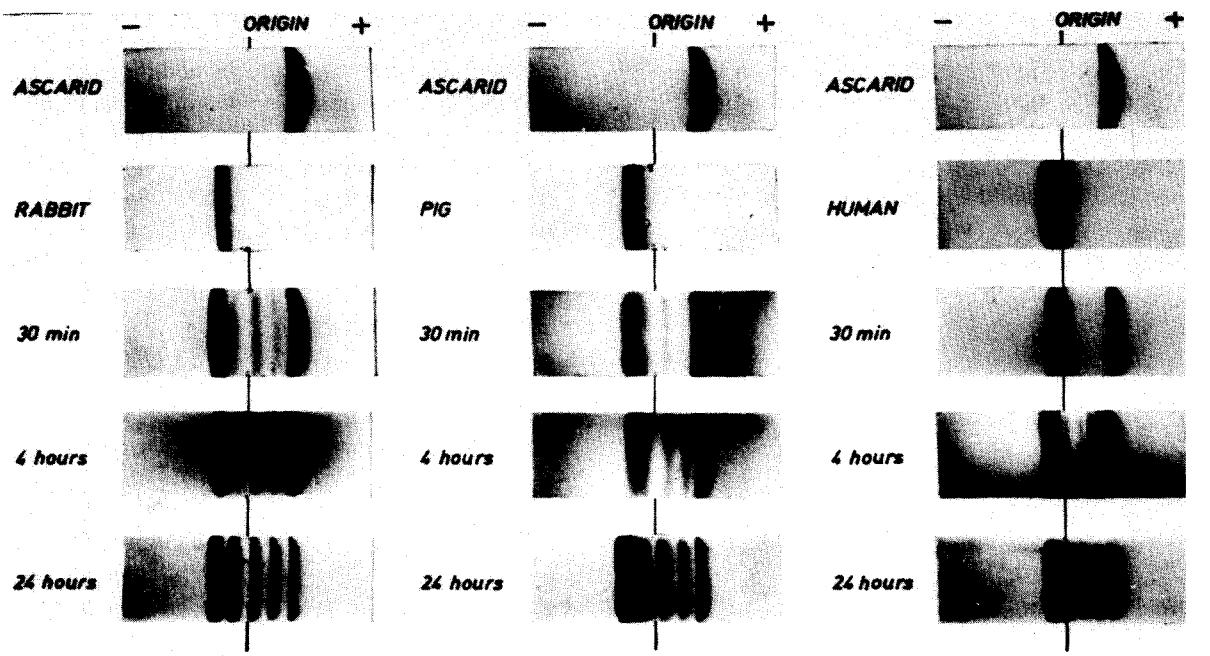
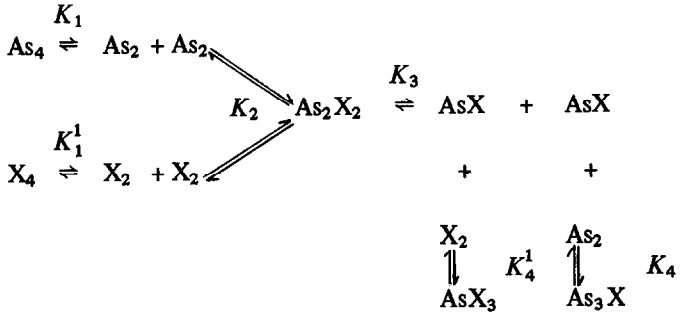


Fig. 1. Hybridization studies. Electrophoretic resolutions of the control samples of ascarid and rabbit, or ascarid and pig, or ascarid and human glyceraldehyde-3-P dehydrogenase are shown on the first two electrophoretic strips of each column. The electrophoretic resolution of an equimolar mixture of ascarid and rabbit glyceraldehyde-3-P dehydrogenase after 30 min, 4 hr, and 24 hr of the subunit exchange reaction is shown in the third, fourth and fifth strips respectively of a left column. The analogous experiments done with ascarid and pig enzyme, and with ascarid and human glyceraldehyde-3-P- dehydrogenase are shown in the middle and right column respectively. The hybridization experiments were performed as described in 'Methods'. Electrophoresis and activity stains were performed as described in 'Methods'.

AsX_3, X_4. The electrophoretic profile of individual control samples revealed only a single electrophoretic band. There is a dissimilarity in the rate of subunit exchange between the ascarid enzyme and those of the human, pig, and rabbit muscles. After 30 min of incubation there are no detectable amounts of hybrid formation between the human and ascarid enzymes, whereas the symmetrical hybrid (As_2P_2) is visible with the ascarid and pig enzymes. However, the entire set has begun to be formed with the ascarid and rabbit enzymes (As_4, As_3R, As_2R_2, AsR_3, R_4) within 30 min. Further experiments over shorter time periods and higher protein concentrations (2–4 mg/ml) indicated that in all cases complete hybridization started from the symmetrical hybrid, As_2X_2. In fact, hybridization studies with protein concentrations of 4 mg/ml or higher yielded only the symmetrical middle hybrid, As_2X_2 in incubation periods as long as 48 hr.

The kinetics of our hybridization studies support the concept that the tetrameric state of glyceraldehyde-3-P dehydrogenase is in rapid equilibrium with the dimers [1, 3, 14]. The symmetrical As_2X_2 hybrid always formed first in each parental combination. The formation of the later-appearing unsymmetrical hybrids (As_3X, AsX_3) may indicate that significant levels of monomers are present in much lower concentrations than the dimers. Conversely, the appearance of unsymmetrical hybrids might not require the presence of monomers and could result from the formation of heterodimers as follows:



This mechanism simply requires that a rapid equilibrium exist between the tetramer and dimer and that the symmetrical hybrid (As_2X_2) is formed first. Following symmetrical hybrid formation, dimerization occurs again, producing heterodimers, AsX. Reassociation with the homodimers (As_2, X_2) would produce the unsymmetrical hybrids, As_3R or AsR_3. Since glyceraldehyde-3-P dehydrogenase has identical subunits [15], this type of subunit interchange is plausible. It is interesting to note that Millar et al. [16] hybridized lactate dehydrogenase under mild conditions and reported that the symmetrical hybrid was the 'dominant heteropolymeric species' in all cases. These results can be interpreted to mean that in LDH the unsymmetrical dimers must form at a slower rate than the homodimer as our results suggest.

That hybridization occurred readily in our system and has required more rigorous conditions in other studies [6, 7] is probably due to the higher pH utilized (near the isoelectric point of the proteins) and the lower protein concentrations employed. We are intensively studying the effects of the borate buffer and other factors on the dissociation and hybridization phenomena.

It might be surprising that the roundworm glyceraldehyde-3-P dehydrogenase and the mammalian enzymes are capable of hybridization, since at first glance they appear to be so phylogenetically distinct from each other. However, the parasite has evolved along with and within the mammal and we have shown [9, 18, 19] that at least two of the enzymes of the glycolytic pathway, aldolase and glyceraldehyde-3-P dehydrogenase, have structures and catalytic properties that are very similar to those of their hosts. Thus, the enzymes of *Ascaris suum* and other parasitic helminths can serve as models for comparing structure and function as well as subunit interactions of various mammalian enzymes.

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